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Review

# The potential of plant systems to break the HIV-TB link

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## Summary

Tuberculosis (TB) and human immunodeficiency virus (HIV) can place a major burden on healthcare systems and constitute the main challenges of diagnostic and therapeutic programmes. Infection with HIV is the most common cause of *Mycobacterium tuberculosis* (Mtb), which can accelerate the risk of latent TB reactivation by 20-fold. Similarly, TB is considered the most relevant factor predisposing individuals to HIV infection. Thus, both pathogens can augment one another in a synergetic manner, accelerating the failure of immunological functions and resulting in subsequent death in the absence of treatment. Synergistic approaches involving the treatment of HIV as a tool to combat TB and vice versa are thus required in regions with a high burden of HIV and TB infection. In this context, plant systems are considered a promising approach for combatting HIV and TB in a resource-limited setting because plant-made drugs can be produced efficiently and inexpensively in developing countries and could be shared by the available agricultural infrastructure without the expensive requirement needed for cold chain storage and transportation. Moreover, the use of natural products from medicinal plants can eliminate the concerns associated with antiretroviral therapy (ART) and anti-TB therapy (ATT), including drug interactions, drug-related toxicity and multidrug resistance. In this review, we highlight the potential of plant system as a promising approach for the production of relevant pharmaceuticals for HIV and TB treatment. However, in the cases of HIV and TB, none of the plant-made pharmaceuticals have been approved for clinical use. Limitations in reaching these goals are discussed.

## Introduction

Since its discovery in 1981 in the United States, human immunodeficiency virus (HIV) had remained a major threat to global health in unprecedented dimensions (Barre-Sinoussi *et al.*, 1983). Despite significant progress in HIV/AIDS research, approximately 37 million people are living with HIV (aidsinfo.unaids.org). HIV has continued to be the leading cause of death in sub-Saharan Africa and was reportedly responsible for the death of 1.1 million people globally in 2015 (aidsinfo.unaids.org). Current predictions suggest that more than 5700 people will become infected with HIV every day, which corresponds to approximately 240 every hour. Moreover, HIV infection can promote the progression of associated diseases, such as tuberculosis (TB) (Zetola *et al.*, 2016). Many resource-limited regions with a high rate of HIV also have a high TB prevalence rate due to lack of screening tests and efficient treatment (Meintjes *et al.*, 2008; Murray *et al.*, 2014). TB and AIDS are high-burden diseases that lead to death worldwide. According to the World Health Organization (WHO), 6.3 million new cases of TB were reported in 2016, which is equivalent to 61% of the estimated prevalence of 10.4 million, and close to 1.7 million deaths from tuberculosis, including 374 000 deaths among people co-infected with HIV, were reported in 2016 (http://apps.who.int/iris/bitstream/handle/10665/259366/9789241565516-eng.pdf;jsessionid=9F217E934CD2D5155BD5180DE2FC75E9?sequence=1). TB is the major single cause of death in regions with HIV (Corbett *et al.*, 2003) and is associated with nearly 26% of AIDS-related deaths (Getahun *et al.*, 2010), 99% of which occur in developing countries (Collins *et al.*, 2002). In this regard, India and South Africa are countries with the highest burden of HIV-associated TB (Department of Health, 2014; World Health Organization, 2016).

The *Mtb*–HIV relationship is attributed both to the suppression of host immune systems by HIV (Schluger and Rom, 1998) and to environmental risk factors allocated by the two diseases (Drobniewski *et al.*, 2005), which complicates their diagnosis and treatment and places an immense burden on public health systems and productivity, particularly in developing countries with large populations of co-infected people (Pawlowski *et al.*, 2012). Both pathogens, HIV and *Mtb*, potentiate the severity of each other's symptoms, increasing the deterioration of the immune system and causing premature death in the absence of treatment. HIV is considered one of the strongest risk factors for the exacerbation of TB infection to active disease and accelerates the risk of latent TB reactivation by 20-fold (Pawlowski *et al.*, 2012), and similarly, TB has been indicated to intensify HIV incidence (Modjarrad and Vermund, 2010).

Although some modest improvements, such as increases in active antiretroviral therapy (ART) coverage and cotrimoxazole preventative therapy, have been obtained among patients with HIV-TB, it remains a complex challenge in extremely high-burden settings. Moreover, co-morbidity decreases the efficiency of both

anti-TB and antiretroviral drugs, and co-administration can augment the risk of drug-related toxicity as well as drug resistance. Because the prevalence of both diseases contributes to the mortality rate in poor countries, it can disproportionately affect people's ability to afford therapy. This is particularly related to the production costs of biopharmaceuticals through expensive fermenter-based systems and further downstream processes (Ma *et al.*, 2003; Stoger *et al.*, 2005) as well as the expensive requirements for cold chain storage and transport (Chan and Daniell, 2015; Kwon *et al.*, 2013a).

Considering the aforementioned facts, efficient alternatives to combat the threat of TB and HIV incidence are urgently required, and in this context, plant system offers potential capacities. This review elaborates on plant molecular pharming and different medicinal plants that are used in the treatment of HIV and TB infection. Because the recent armamentarium against HIV and TB exhibits remarkable limitations ranging from toxicity to extensive and expensive therapy, the potential of plants in terms of safety and cost-effective expression platforms as well as the production of plant natural compounds with anti-TB and HIV potential will contribute to further eradication and management of HIV and TB, especially in extremely high-burden settings. The knowledge obtained regarding the use of molecular pharming and medicinal plants in HIV and TB treatment as well as the promising results obtained in different studies shows that plants are efficient, convenient and affordable platforms for the production of biopharmaceuticals for use as an immunomodulatory agent or a supplement for the treatment of TB and HIV as well as HIV-TB co-infection. However, in the cases of HIV and TB, none of the plant-made pharmaceuticals have been approved for clinical use. Limitations in reaching these goals are discussed.

## Routes of HIV and TB infection

HIV transmission is initiated by sexual relationships via virus exposure at the mucosal epithelium of the vagina or rectum, by maternal–infant exposure and by percutaneous inoculation (Shaw and Hunter, 2012). However, the precise transmission mechanism is complicated and has not been fully elucidated, and different strategies are involved. It is globally understood that macrophages, T cells and dendritic cells are favourable destinations for HIV. HIV invades the CD4 cells of the immune system and employs the machinery of the CD4 cells to multiply and spread throughout the body. Virus protein gp120 binding to the CD4 receptor initiates the cascade of conformational changes in gp 120 that enable gp120 to interact with the host cell co-receptor. Engagement of the co-receptor induces a further conformational change in the envelope glycoprotein that leads to the induction of gp41-mediated virus–host cell membrane fusion. The virus progressively starts its infection through transformation of the viral RNA genome into a pro-viral DNA. The integrated provirus is transcribed by the host RNA polymerase II into full-length viral RNA, which can subsequently be individually or multiple spliced (Shors, 2011). The synthesized viral RNA is then transported from the nucleus to the cytoplasm for translation to gRNA for packaging. The host cellular machinery generally inhibits the transport of single and unspliced viral RNA species, and to overcome this obstacle, HIV-1 synthesizes the Rev protein, which binds to the Rev response element (RRE) and to the chromosomal maintenance 1 (CRM1)/RanGTP nuclear export complex (Mailler *et al.*, 2016). After export from the nucleus and mRNA translation, a proteolytic processing undertaken by the viral protease that occurs during or quickly following the budding of the immature particle (Konnyu *et al.*, 2013) leads to synthesis of the structural proteins that are critical for morphological rearrangements of the viral particle (Bell and Lever, 2013) (Figure 1).

TB is an airborne infection caused by the bacillus *Mtb,* which is transmitted via the inhalation of infectious droplet nuclei carried by the fluids expelled by coughing, sneezing or shouting individuals infected with pulmonary or laryngeal TB disease (https://www.cdc.gov/tb/education/corecurr/). Transmission occurs when droplet nuclei containing *Mtb* are inhaled by an individual, and the droplet nuclei pass from the mouth, the nasal and upper respiratory tract, and bronchi to arrive at the alveoli of the lungs (https://www.cdc.gov/tb/education/corecurr/). Professional phagocytic cells in the lungs, such as dendritic cells, macrophages, monocytes and neutrophils, can be recruited by the progressive growth of the mycobacterial population and develop into early granulomas (Kang *et al.*, 2011; Wolf *et al.*, 2007).

Obtaining an in-depth understanding of the immune response against *Mtb* is a major challenge to the lives and health of susceptible human populations. Based on this challenge, the available evidence indicates that although most humans experimentally establish sufficient immune responses after infection, these immune responses do not completely eliminate the bacteria. However, immunological, epidemiological and genetic studies have demonstrated that both T-cell-mediated immunity and innate immunity are involved in tuberculosis, and different T-cell subsets, cellular receptors and humuoral factors are required (van Crevel *et al.*, 2002).

## Drugs used to combat HIV and TB co-infection

The emergence of highly ART has remarkably decreased both the mortality and the morbidity caused by HIV (Hogg *et al.*, 1998). Among HIV-infected people, TB is considered one of the most potent infections, leading to death in resource-limited areas (Corbett *et al.*, 2003). Although both ART and anti-tubercular treatment (ATT) have been demonstrated to increase the survival of co-infected individuals, establishing a precise ART regimen remains controversial due to many concerns of unfavourable drug interactions.

Efavirenz and nevirapine, which are classes of non-nucleoside reverse transcriptase inhibitors (NNRTIs), are generally recommended for individuals infected with HIV and TB. Rifamycins, especially rifampicin (RMP), in combination with isoniazid (INH), ethambutol (EMB) and pyrazinamide (PZA), are key medications for the treatment of TB because RMP is a potent inducer of the cytochrome P450 enzyme system, which leads to improved clearance of NNRTIs (Jiang *et al.*, 2014). Rifamycins provide inducing effects on phase I and II drug-metabolizing enzymes and transporters (Rabie *et al.*, 2017). However, ART is often metabolized by a rifamycin-induced cytochrome P450 enzyme system or might prevent specific enzyme activity caused by drug–drug interactions with rifamycins, which may lead to adverse modifications in the pharmacokinetic and pharmacodynamic characteristics of the drug. Drug interactions can modify the absorption, transport, distribution, metabolism or excretion of drug which may result in viral resistance or serious toxic effects leading treatment failure (Abdool Karim *et al.*, 2010). In this context, the emergence of viral resistance to antiretroviral (ARV) drugs is a common cause of ART failure.

 

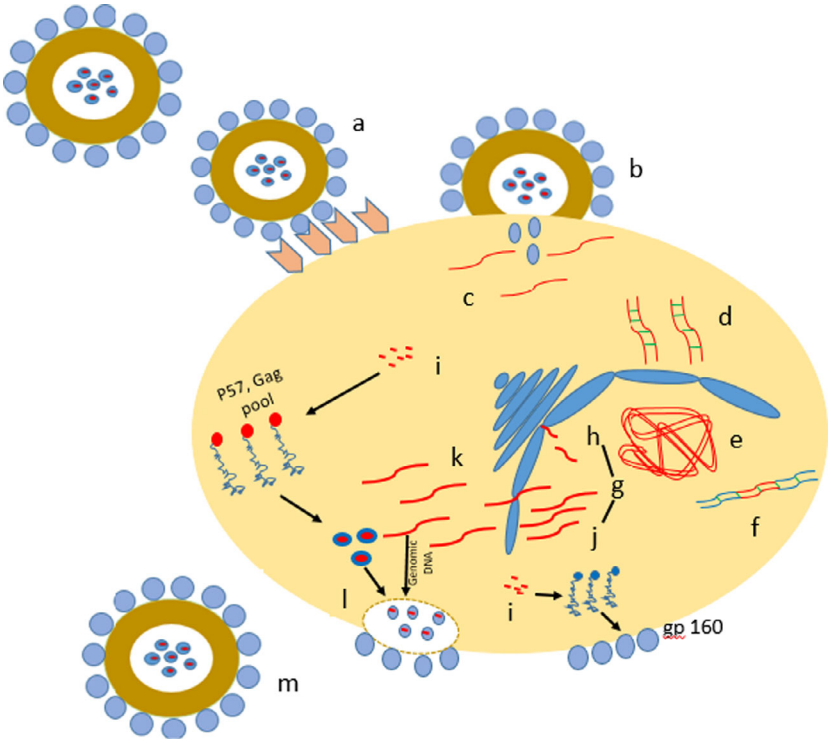


**Figure 1** schematic presentation of HIV life cycle. (a) HIV attachment to CD4 antigen and a specific chemokine receptor. (b) Virus fusion with the cell membrane and entry of the virion core into the cell. (c) Release of viral RNA and core proteins and their transport into the nucleus. (d) Formation of double-stranded DNA by reverse transcriptase. (e) Transport of double-stranded viral DNA into the cell nucleus. (f) Integration of viral DNA into cellular DNA. (g) Synthesis of viral RNA by RNA polymerase II and production of RNA transcripts with shorter spliced RNA (h) and full-length genomic RNA (j). (h) Transport of shorter spliced RNAs to the cytoplasm and the production of several viral proteins that are then modified in the Golgi apparatus of the cell (i). (j) Transport of full-length genomic RNAs into the cytoplasm (k). (l) Assembly, budding and maturation of new virions. (m) Release of mature virus.

## Natural sources of plant metabolites with anti-HIV and TB activity

According to archaeological excavations, it is likely that the humans recognized and used medicinal plant as long as 60 000 years ago (Fabricant and Farnsworth, 2001), representing the potential of plant medicine in production of valuable cocktail secondary metabolites for our healing and health care (Sharifi-Rad *et al.*, 2018). Additionally, based on this record we can say that humans have been involved for thousands of years in a vast 'clinical trial' with medicinal plants as they have tested their efficacy. Moreover, historically, oral administration of plant-made medicines through direct using of plant tissues or by the preparation of crude extracts considered as a great advantages as these strategies are both simple and inexpensive as well as remove disadvantages related to syringe-based injection or other required invasive procedures.

Plant secondary metabolites are involved in the adaptation of plants to their environment, and most of these metabolites are involved in plant defence mechanisms against pathogen invasion and abiotic stress (Habibi *et al.*, 2017b). These valuable compounds could be extracted from different parts of plants, such as leaves, shoots, roots and flowers, and investigated for their potential as anti-HIV and anti-TB drugs. Phenolic compounds, flavonoids, triterpenes, coumarins and alkaloids are examples of prominent natural products with anti-HIV and TB activity (Kurapati *et al.*, 2016). A condensed summary of the most active plant secondary metabolites against HIV and TB is provided in Tables 1 and 2, respectively.

### Medicinal plants for the treatment of AIDS

Although antiretroviral therapy allows patients living with HIV to live longer and maintain their HIV symptoms at undetectable levels, the cost of the medications used in highly active antiretroviral therapy (HAART) is especially high for those living in developing countries, and these medications also have other drawbacks, such as viral reservoirs and drug resistance due to lifelong use, side effects, including nausea, headaches, poor appetite and stomach cramps, and severe toxicities, such as cardiovascular disease, neurotoxicity, kidney disease, hypersensitivity reactions, liver damage, insulin resistance, diabetes and body fat redistribution (Williams-Orlando, 2017). These disadvantages of HAART have encouraged a search for new better (safe, effective and cheap) anti-HIV agents in plant species.

Medicinal plants offer a number of advantages that make them a promising alternative to antiretroviral therapy or as a complementary treatment to decrease the drawbacks associated with HAART (Salehi *et al.*, 2018). In comparison with HAART, medicinal plants are more attractive because they are less expensive and exhibit low side effects. Medicinal plants block HIV at various stages of the viral life cycle. Some of these plants decrease viral entry into plasma, improve immune function and boost the quality of life, and if administered alone or in combination with highly active antiretroviral therapy, some plants relieve the side effects of biomedical drugs (Chu and Liu, 2011), potentiate an antioxidant status, augment or sustain CD4 T cells, reduce chronic inflammation and boost neuroprotection (Williams-Orlando, 2017). Several anti-HIV herbs are being investigated in clinical trials (Table 3) or are being used for the treatment of individuals infected with HIV.

Considering the above advantages, the World Health Organization (WHO) has strongly recommended the evaluation of ethno-medicines and other natural compounds for anti-HIV activity because they offer efficient and tremendous therapeutic properties (WHO, 1989a,b). In this context, those of compounds which interfere with different stage of HIV virus are ideal. The natural agents including calanolides (coumarins), baicalin (flavonoid), polycitone A (alkaloid), ursolic and betulinic acids

**Table 1** Summarized plant active compounds against HIV

| Compound group | Agent | Mechanism of action | $EC_{50}$ | $IC_{50}$ | Plant system | Reference |
|---|---|---|---|---|---|---|
| Alkaloids | Papaverine | Inhibits HIV replication and decreases HIV protein production | – | 10 μg/mL | *Papaver somniferum L. (Papaveraceae)* | Turano *et al.* (1989) |
| | Emetine | Inhibits HIV-1 replication by interfering with reverse transcriptase activity | 0.03 μM | – | *Psychotria ipecacuanha* | Chaves Valadao *et al.* (2015) |
| | Corydine | Inhibits HIV-1 reverse transcriptase enzyme activity | | 356.8 μg/mL | *Croton echinocarpus* | Ravanelli *et al.* (2016) |
| | Norisoboldine | | | 53.7 μg/mL | | |
| | Buchapine | Inhibits the cytopathogenic effects of HIV-1 | 0.94 μM | – | *Evodia roxburghiana* | McCormick *et al.* (1997) |
| | Matairesinol | Inhibits HIV replication | 0.037 μM | – | *Symplocos setchuenensis* | Ishida *et al.* (1997) |
| | Harman | | 210 μM | – | | |
| | O-Demethylbuchenavianine | Not defined | | 0.66 μM | *Buchenavia capitata* | Beutler *et al.* (1992) |
| | Nitidine chloride | Inhibits HIV-1 reverse transcriptase enzyme activity | – | 49 μg/mL | *Toddalia asiatica* | Tan *et al.* (1991) |
| | Fagaronine chloride | | – | 10 μg/mL | | |
| | Schumannificine | Irreversibly binds to gpl20 | 1.6 μM | | *Schumanniophyton magnificum* | Houghton *et al.* (1997) |
| Coumarins | Suksdorfin | Inhibits HIV replication | 2.6 ± 2.1 μM | | *Lomatium suksdorfii (S. Watson) J.M.* | Lee *et al.* (1994) |
| | Calanolide A | Inhibits HIV replication | – | 0.32 μM | *C. cordato-oblongum* | Dharmaratne *et al.* (1998) |
| | Cordatolide A | Inhibits HIV replication | – | 19.3 μM | *C. cordato-oblongum* | Dharmaratne *et al.* (1997) |
| | Cordatolide B | | – | 11.7 μM | | |
| Terpenes | Uvaol | Inhibits HIV-1 protease | – | 5.5 μM | *Crataegus pinnatifida* | Min *et al.* (1999) |
| | Ursolic acid | | – | 8 μM | | |
| | Maslinic acid | Inhibits HIV-1 protease | – | – | *Geum japonicum* | Xu *et al.* (1996) |
| | Celasdin B | Anti-HIV replication activity | – | 0.8 μg/mL | *Celastrus hindsii* | Kuo and Kuo (1997) |
| | Garciosaterpene A Garciosaterpene B | Inhibits HIV-1 reverse transcriptase | – | 5.8 μg/mL | *Garcinia speciose* | Rukachaisirikul *et al.* (2003) |
| | Suberosol | Not defined | 33 μg/mL | – | *Polyalthia suberosa* | Li *et al.* (1993) |
| | Tripterifordin | Anti-HIV replication activity | 1 μg/mL | – | *Tripterygium wilfordii* | Chen *et al.* (1992) |
| | Lancilactone C | Inhibits HIV replication | 1.4 μg/mL | – | *Kadsura lancilimba* | Chen *et al.* (1999) |
| Flavonoid | Baicalin | Anti-HIV-1 activity as a non-nucleoside reverse transcriptase inhibitor | | | *Scutellaria baicalensis* | Li *et al.* (2000) |
| | 6,8 Diprenylaromadendrin | Anti-HIV activity in an XTT-based, whole-cell screen | 2.1 μg/mL | 4.7 μg/mL | *Monotes africanus* | Meragelman *et al.* (2001) |
| | 6,8-Diprenylkaempferol | | 2.4 μg/mL | 5.8 μg/mL | | |
| | Lonchocarpol A | | 1.3 μg/mL | 2.7 μg/mL | | |
| | Quercetin 3-O-(2-galloyl) a-L-arabinopyranose | Inhibit integrase activity of HIV-1 | – | 18.1 μg/mL | *Acer okamotoanum* | Kim *et al.* (1998) |
| | Morelloflavone | Inhibition of the polymerase of HIV-1 | 6.9 μM | – | *Rhus succedanea* | Lin *et al.* (1997) |
| | Robustaflavone | | – | 65 μM | | |
| | Hinokiflavone | | – | 65 μM | | |
| | Alnustic acid methyl ester | Inhibition of HIV-1 protease | 15.8 μM | | *Alnus firma* | Yu *et al.* (2007) |
| | Quercetin | Inhibition against HIV-1 reverse transcriptase | – | 60 μM | *A. firma* | Yu *et al.* (2007) |
| | Quercitrin | | – | 60 μM | | |
| | Myricetin 3-O-beta-D-galactopyranoside | | – | 60 μM | | |
| | Xanthohumol | Induce cytopathic effects, the production of viral p24 antigen | 20.74μ/mL | – | *Hops Humulus lupulus* | Wang *et al.* (2004) |
| Phenolics | Lithospermic acid | Integrase inhibitors | – | 2 μM | *Salvia miltiorrhiza* | Abd-Elazem *et al.* (2002) |
| | Lithospermic acid B | | – | 6.9 μM | | |
| | Corilagin, quercetin | Inhibitory against human immunodeficiency virus (HIV) reverse transcriptase | – | 20 μM | *Chamaesyce hyssopifolia* | Lim *et al.* (1997) |
| | 3-*O* -β-D-glucopyranoside | | – | 50 μM | | |

 

**Table 1** Continued

| Compound group | Agent | Mechanism of action | $EC_{50}$ | $IC_{50}$ | Plant system | Reference |
|---|---|---|---|---|---|---|
| | 1,3,4,6-tetra-*O* -galloyl-β-D-glucopyranose | | – | 86 μM | | |
| | Repandusinic acid | Inhibition of HIV-1 reverse transcriptase | – | 2.5 μM | *Phyllanthus niruri* | Ogata *et al.* (1992) |
| | Monopotassium salt of isomeric caffeic acid | Inhibition of HIV replication | 2.8 μg/mL | – | *Arnebia euchroma* | Kashiwada *et al.* (1995) |
| | Monosodium salt of isomeric caffeic acid | | 4 μg/mL | – | | |
| | Vismiaphenone D | Inhibition of the HIV-1 protease | 11 μg/mL | | *V. cayennensis* | Fuller *et al.* (1999) |
| | Peltatol A | Not defined | – | 35 μg/mL | *Pothomorphe peltata* | Gustafson *et al.* (1992) |
| Lignans | Kadsulignans M | Inhibition of HIV reverse transcriptase activity | $6.03 \times 10^{-6}$ M | $1.19 \times 10^{-4}$ M | *K. Coccinea* | Liu and Li (1995) |
| | Olignan A | HIV-1 reverse transcriptase (RT) inhibitory | – | 60.4 μg/mL | *Anogeissus acuminata* | Rimando *et al.* (1994) |
| | Olignan B | | – | 1.072 μg/mL | | |
| | Gomisin | Inhibitors of HIV replication | 0.006 μ/mL | – | *K. interior* | Chen *et al.* (1997) |
| | (+/-)-Gomisin M1 | Inhibitory against HIV replication | 0.65 μM | 44.5 μM | *Schisandra rubriflora* | Chen *et al.* (2006) |
| | Rubrisandrins A | | 11.3 μM | >64 μM | | |
| | Schisanhenol | | 5.7 μM | 42 μM | | |
| | Wilsonilignan A | Preventing the cytopathic effects of HIV-1 | 3.26 μg/mL | – | *S. wilsoniana* | Yang *et al.* (2010) |
| | Wilsonilignan B | | 6.18 μg/mL | – | | |
| | Wilsonilignan C | | 2.87 μg/mL | – | | |
| Aponins | Actein | Activity against HIV replication | 0.375 μg/mL | – | *Cimicifuga racemosa* | Sakurai *et al.* (2004) |
| Sulphated polysaccharide | Prunellin | Interfere with HIV-1 virion binding to permissive cells by blocking CD4 interactions | – | 1.1 μg/mL | *Prunella vulgaris L* | Oh *et al.* (2011) |

(triterpenes) and lithospermic acid (phenolic compound) have indicated efficient activity against HIV; however, most of these investigations are in vitro, and a too few investigations have been carried out in vivo or in human studies (Salehi *et al.*, 2018). Different assays and protocols have been developed for preliminary screening of natural compounds against HIV. HIV-1 replication inhibition assays could be performed with simple cell-based assays including MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Weislow *et al.*, 1989) or XTT: sodium 3-[1-(phenylamino)-carbonyl]-3,4-tetrazoliumbis(4-methoxy-6-nitro) benzene-sulphonic acid hydrate assays (Mahmood, 1995) and HIV p24 expression using human T-cell lines (Lee-Huang *et al.*, 1991). Target specific analysis could be carried out by assessment of compound effect on HIV gp120/CD interaction, reverse transcriptase inhibition assay (using radio-labelled nucleotide, non-radioactive ELISA method and RNase H activity), integrase inhibition assay (using radio-labelled oligonucleotide substrate or by using non-radioactive ELISA) and protease inhibition assay by specific techniques such as ELISA, liquid scintillation counting, fluorometric, spectrophotometric and HPLC-based methods. However, further pharmacological and toxicological analyses of crude extract and/or isolated compound are required.

As described above, the concomitant use of medicinal plants with conventional therapy is a promising approach to relieve the adverse side effects of antiretroviral therapy and to improve the therapeutic properties of the administered drugs. In this context, there are considerable reports on the co-administration of herbal medicine with conventional therapy. Huang *et al.* (2007) reported that resin components of *Dracaena cochinchinensis* can relieve the adverse reaction of HAART drugs and boost the efficacy of HAART. Zhao *et al.* (2006) demonstrated the enhancing effects of TCM on HAART, which involve inhibition of viral rebound and maintenance of immune functions after HAART is stopped. These researchers also indicated a significant improvement in a range of symptoms and signs associated with HAART, such as diarrhoea, fatigue, nausea, skin rash and anorexia. Additionally, the co-administration of herbal medicine with antiretroviral drugs could adversely enhance drug bioavailability and therapeutic properties by modifying the absorption of the co-administered drug (Brown *et al.*, 2008). In this context, interference of the active transport process during efflux by the co-administered medicinal plant is considered a factor that may affect drug absorption (Balayssac *et al.*, 2005). Brown *et al.* (2008) indicated that water extracts of *Hypoxis hemerocallidea* and l-canavanine (a constituent of *Sutherlandia frutescens*), when taken concomitantly, remarkably potentiate the bioavailability of nevirapine by improving the transport of nevirapine across human intestinal epithelial cells.

However, the use of medicinal plants concomitantly with HAART should be further clinically evaluated because there is a potential risk of herb–drug interaction (HDI) (Chen *et al.*, 2011), which may significantly exacerbate the health status of the patients if not detected early during treatment. Despite the wide use of medicinal plants among patients being administered

**Table 2** Summary plant agents with potent activity against different strains of *Mtb*

| Type of inhibitor | Compound family | Compound name | Mechanism of action | MIC | Plant species | Plant part used | Reference |
|---|---|---|---|---|---|---|---|
| MDR inhibitor | Alkaloid | Aristolactam I | Unknown | 12.5 and 25 μg/mL | *Aristolochia brevipes* | Rhizome | Navarro-García *et al.* (2011) |
| MDR inhibitor | Alkaloid | Tiliacorinine, 20, nortiliacorinine, tiliacorine | Inhibits RNA and protein synthesis | 3.1 μg/mL | *Tiliacora triandra* | Root | Sureram *et al.* (2012) |
| MDR inhibitor | Alkaloids | Vasicine acetate<br>2-Acetyl benzylamine | Unknown | 200 μg/mL<br>50 μg/mL | *Adhatoda vasica Ness* | Leaves | Ignacimuthu and Shanmugam (2010) |
| MDR/XDR-TB | Alkaloid | (−)-Deoxypergularine (DPX) | Inhibits growth | 12.5 μg/mL | *Cynanchum atratum* | Root | Nam *et al.* (2016) |
| MDR inhibitor | Cinnamic acid | Ethyl p-methoxycinnamate | Inhibits the growth of Mtb | 0.242–0.485 Mm | *Kaempferia galanga L.* | Rhizome | Lakshmanan *et al.* (2011) |
| MDR inhibitor | Diterpene | Abietane and its derivatives | Interacts with the NADPH-dependent enzyme mycothiol disulphide reductase of Mtb and/or causes cell damage induced by the formation of radical onions and reactive oxygen species | 3.1–3.9 μg/mL | *Plectranthus grandidentatus* | Aerial parts | Rijo *et al.* (2010) |
| MDR inhibitor | Furanocoumarin | 2,4-Undecadienal | Shows reactivity against nucleophilic groups (amino, hydroxy, sulfhydryl) in proteins associated with the cell membrane | 25–50 μg/mL | *Foeniculum vulgare Mill.* | Stems and leaves | Esquivel-Ferriño *et al.* (2012) |
| MDR inhibitor | Lignan | Fargesin, (8R,8′R,9R)-cubebin | Unknown | 25–50 μg/mL | *A. elegans Mast.* | Rhizome | Jiménez-Arellanes *et al.* (2012) |
| MDR inhibitor | Lignan | Licarin A | Unknown | 3.12–12.5 μg/mL | *A. taliscana* | Root | León-Díaz *et al.* (2010) |
| | Lignan<br>Lignan | Beilschmin A<br>Beilschmin B | Unknown | 2.5 μg/mL<br>7.5 μg/mL | *Beilschmiedia tsangii* | Leaves | Chen *et al.* (2007) |
| MDR inhibitor | Lignan<br>Lignan<br>Lignan<br>Flavonoid | 3′-Demethoxy-6-O-demethylisoguaiacin,<br>Dihydroguaiaretic acid<br>4-Epi-larreatricin<br>5,4′-Dihydroxy-3,7,8,3′-tetramethoxyflavone, 5,4′-dihydroxy-3,7,8-trimethoxyflavone | Unknown | 12.5 μg/mL<br>12.5–50 μg/mL<br>25 μg/mL<br>25 μg/mL<br>25–50 μg/mL | *Larrea tridentata* | Leaves | Favela-Hernandez *et al.* (2012) |
| MDR inhibitor | Quinone | Diospyrin | Unknown | 100 μg/mL | *Euclea natalensis* | Root | Lall and Meyer (2001) |
| MDR/XDR | Triterpene<br>Quinone | Ursolic acid<br>Hydroquinone | Inhibits the biosynthesis of mycolytic acid involved in *Mtb* cell wall formation<br>Disrupts intracellular components such as DNA, RNA, internal proteins and other organelles of *Mtb* | 12.5–25 μg/mL | *A. capillaris* | No report | Jyoti *et al.* (2016) |
| MDR inhibitor | Monoterpenoid | Plumericin<br>Isoplumericin | Unknown | 1.3–2.1 μg/mL<br>2–2.6 μg/mL | *Plumeria bicolor* | Stem bark | Kumar *et al.* (2013) |
| MDR inhibitor | Phenol | Mono-O-methylcurcumin isoxazole (analog) | Unknown | 0.195-3.125 μg/mL | *Curcuma longa L* | Rhizomes | Changtam *et al.* (2010) |

 

**Table 2** Continued

| Type of inhibitor | Compound family | Compound name | Mechanism of action | MIC | Plant species | Plant part used | Reference |
|---|---|---|---|---|---|---|---|
| MDR inhibitor | Sesquiterpenoids | 1a-Acetoxy-6b, 9b-dibenzoyloxydihydro-b-agarofuran | Inhibits the growth of *Mtb* | 6.2 μg/mL | *C. vulcanicola* | Leaves | Torres-Romero *et al.* (2011) |
| MDR inhibitor | Triterpenoid | Azorellane<br>Azorellanol | Unknown | 12.5 μg/mL | *Azorella compacta Phil*<br>*A. madreporica Clos.* | Aerial parts | Molina-Salinas *et al.* (2010) |
| MDR inhibitor | Triterpenoid | 25-Hydroperoxycycloart-23-en3β-ol | Induces oxidative stress by lipid peroxidation, protein oxidation or nucleic acid damage | 12.5 μg/mL | *Blepharodon nitidum* | Whole plant | Aponte *et al.* (2008) |
| MDR inhibitor | Triterpenoid | Cucurbitacin E 2-0-β-D-glucopyranoside<br>Ursolic acid | Involved in the formation of pores in the lipid bilayer of the bacterial cell wall<br>Disrupts the *Mtb* membrane | 125 μg/mL<br>50 μg/mL | *Citrullus colocynthis (L.) Schrad.* | Fruit | Mehta *et al.* (2013) |
| MDR inhibitor | Triterpenoid | Aegicerin | Unknown | 1.6- 3.12 μg/mL | *Clavija procera* | Stems and bark | Rojas *et al.* (2006) |
| MDR inhibitor | Triterpenoid | 3-acetoxy-22-(2 -methyl2Z-butenyloxy)-12-oleanen-28-oic acid,<br>3-hydroxy-22-(2 -methyl-2Z-butenoyloxy)-12-oleanen-28-oic acid,<br>oleanolic acid | Inhibits the growth of *Mtb* | 50 μg/mL<br>50 μg/mL<br>25 μg/mL | *Lantana hispida* | Aerial parts | Jimenez-Arellanes *et al.* (2007) |
| Reduction in intracellular viability | Alkaloids | Decarine | Unknown | 1.6 μg/mL | *Zanthoxylum capense* | Root | Luo *et al.* (2013) |
| | Flavonoids | Myricetin<br>Quercitin-3-O-β-D-glucoside | Unknown | 0.25 μg/mL<br>0.25 μg/mL | *Pelargonium reniforme* | Root | Kim *et al.* (2009) |
| | Phenol | 7-Methyljuglone | Intracellularly penetrates into macrophages | 0.5 μg/mL | *E. natalensis* | Root | Lall *et al.* (2005) |
| | Phenol | 9-Epigallocatechin-3-gallate | Downregulates tryptophan-aspartate containing coat protein (TACO) gene transcription within human macrophages through its ability to inhibit the Sp1 transcription factor | - | *Camellia sinensis* | Leaves | Anand *et al.* (2006) |
| | Triterpene | Ursolic acid<br>Oleanolic acid | Restores the protective antimycobacterial cytokine pattern by modulating TGF-β and cyclooxygenase type 2 (COX-2) activity, producing a significant decrease in bacillus loads, and tissue damage. | 6.25 μg/mL<br>12.25 μg/mL | *Chamaedorea tepejilote* and *Lantana hispida* | Aerial parts | Jiménez-Arellanes *et al.* (2013) |
| Dormant bacilli | Alkaloid | Chelerythrine | Decreases isocitrate lyase gene expression, which is involved in *Mtb* viability during dormancy | 4 μg/mL | *Chelidonium majus* | Root | Liang *et al.* (2011) |

 

**Table 2** Continued

| Type of inhibitor | Compound family | Compound name | Mechanism of action | MIC | Plant species | Plant part used | Reference |
|---|---|---|---|---|---|---|---|
| | Ester | 1′-Acetoxychavicol acetate | Acts on the genome of *Mtb* to render the bacterium sensitive to antibiotics | 50–100 μg/mL | *Alpinia galanga* | Rhizome | Gupta *et al.* (2014) |
| | Pyrithione | Dipyrithione | Unknown | <0.039 μg/mL | Marsypopetalum modestum (Pierre) B. Xue & R.M.K | Root, stems | Elkington *et al.* (2014) |
| | Terpenoid | Totarol | Unknown | 23.3 μg/mL | *Juniperus communis L* | Aerial parts and root | Gordien *et al.* (2009) |
| | | Longifolene | | 93.4 μg/mL | | | |
| Immunomodulatory activity | Triterpene | Ursolic acid | Increases the expression of IFN-c and TNF-a in the lungs | 6.25 μg/mL | *C. tepejilote* and *Lantana hispida* | Aerial parts | Jiménez-Arellanes *et al.* (2013) |
| | | Oleanolic acid | | 12.25 μg/mL | | | |
| | Phenol | Curcumin | Induces caspase-3-dependent apoptosis and autophagy via inhibition of nuclear factor-kappa B (NFκB) activation | >50 μM | *C. longa* | Root | Bai *et al.* (2016) |

MIC, minimal inhibitory concentration.

HAART, the available information on their pharmacokinetic and pharmacodynamic properties is scarce (He *et al.*, 2011; Thomford *et al.*, 2015), and further studies evaluating these properties in humans are imperative. There is substantial evidence indicating that the co-administration of herbal medicine with conventional therapy results in clinical complications due to HDIs (Chen *et al.*, 2011; Huang *et al.*, 2008; Thomford *et al.*, 2015). Although the prediction of drug interactions during drug development is challenging, the FDA guidelines, methodologies and recommendations related to drug interactions, particularly cytochrome P450-based and transporter-based drug interactions, could be effective (Huang *et al.*, 2008). The FDA's current understanding of drug interactions is available online (http://www.fda.gov/cder/drug/drugInteractions/default.htm). Moreover, to decrease HDIs, phytochemical profiling and subsequent mechanistic studies of the co-administered medicinal plants should be performed to identify the compounds involved in HDIs. Equally important is the regulation of effective drug dose in plant materials or formulations. Standardization of plant medicinal formulations is a key factor in order to analyse drug quality, based on the concentration of their active principles, physical, chemical, phytochemical, standardization and in vitro, in vivo parameters. Due to genetic environmental and cultural factors, the ingredients variability occurs in herbs or herbal preparations which make the use of herbal medicines more complicated. For instance, the availability and quality of the raw materials are frequently challenging, the active principles are diverse or may be unknown, and quality of different batches of preparation may be difficult to control and ascertain (Folashade *et al.*, 2012). Moreover, there is no official regulatory standard for cultivation, preparation of herbal medicines and clinical evaluation.

## Medicinal plants and their secondary metabolites for treatment of TB

Despite remarkable progress in combating TB prevalence, the disease is continuing to spread worldwide, which has led to serious global health challenges: more than 10.4 million patients living with TB and 1.8 million deaths were reported in 2015 (Nguyen, 2016; WHO, 2015, 2016). The continued incidence of TB stems from the fact that the pathogen can survive against host defence mechanisms due to induced multidrug resistance (MDR-TB), extensive drug resistance (XDR-TB) and total drug resistance associated with the inappropriate use of anti-TB medications during treatment for other diseases, such as diabetes mellitus and HIV (WHO, 2015). MDR-TB strains are strongly persistent against both isoniazid and rifampicin medication and caused over 480 000 new infections and 210 000 deaths in 2013 (Nguyen, 2016). XDR-*Mtb* strains are also resistant to quinolones and other second-line drugs because some clinical strains are resistant to all available antibiotics (Adhvaryu and Vakharia, 2011; Mishra *et al.*, 2015). In this case, their morbidity is rapidly increasing in regions with high HIV prevalence.

Under these circumstances, the discovery and production of newer anti-tuberculosis compounds that potentially eliminate antibiotic resistance in terms of MDR-TB and XDR-TB and boost the immune system against *Mtb* is needed to tackle global TB prevention. Moreover, the development of new agents that target the establishment of mycobacterial dormancy in human macrophages or prevent bacterial virulence factors that interfere with the signalling pathways of host cells and affect immunity and cause pathogen resistance should be considered the main future research directions (Koul *et al.*, 2011).

**Table 3** Ongoing and completed clinical trials of medicinal plants for their anti-HIV activity

| Medicinal plant and country | Study design | Purpose | Study phase | Recruitment status | ClinicalTrials.gov number |
|---|---|---|---|---|---|
| *Hypoxis obtuse*, USA | 18 participants, single group assignment, pharmacokinetics single-arm, open label | To evaluate drug interactions between an African potato (*Hypoxis obtuse*) and the antiretroviral agents lopinavir/ritonavir (Kaletra®). | Phase I | Completed | NCT01227590 |
| *A. annua L* and *Moringa oleifera*, Uganda | 282 participants, randomized, double (participant, care provider), parallel assignment. | To study the effect of *A. annua* L and *Moringa oleifera* leaf powder on CD4 cell count and other immunological indices in HAART-treated HIV patients. | Not applicable | Not yet recruiting | NCT03366922 |
| *Echinacea purpurea*, Spain | 15 participants, non-randomized, single group assignment, two arms, open label. | To evaluate drug interactions between *E. purpurea* and the protease inhibitor etravirine. | Phase I | Completed | NCT01347658 |
| *E. purpurea*, Spain | 15 participants, non-randomized, single group assignment, two arms, open label. | To assess drug interactions between one medicinal herb widely used by HIV-infected patients: *Echinacea* sp. and the protease inhibitors darunavir/ritonavir. | Phase 4 | Completed | NCT01046890 |
| *Moringa leifera*, Zimbabwe | 19 participants, observational model: case-crossover, time perspective: prospective. | To characterize the interaction between herbal *M. oleifera* and the antiretroviral drugs nevirapine and efavirenz. | - | Completed | NCT01410058 |
| Traditional Chinese Medicine, USA | 40 participants, parallel assignment. | To compare traditional Chinese medicine with standard antibiotic therapy consisting of pseudoephedrine (Sudafed) plus the amoxicillin/clavulanate potassium combination (Augmentin) in reducing symptoms and recurrence of acute HIV-related sinusitis. | Not applicable | Completed | NCT00002149 |
| *Triptolide wilfordii*, China | 15 participants, non-randomized single group assignment, two arms, open label. | To evaluate the effects and side effects of ARV treatment in Chinese patients in acute HIV-1 infection and to evaluate the impact of *T. wilfordii* on the HIV-1 reservoir. | Phase 3 | Unknown | NCT02219672 |
| *T. wilfordii*, China | 150 participants, randomized, crossover assignment, two arms. | To evaluate the impact of *T. wilfordii* on T-cell immune activation and inflammation biomarkers in HIV-infected immunological non-responders. | Phase 1 and Phase 2 | Unknown | NCT01817283 |
| *T. wilfordii*, China | 300 participants, randomized, parallel assignment, two arms, double-blinded, placebo-controlled study. | To explore the efficacy and safety of *T. wilfordii* on viral suppression, immune recovery and immune activation biomarkers in the treatment of patients with naïve HIV-1 infection. | Phase 3 | Not yet recruiting | NCT03403569 |
| *Euphorbia kansui*, USA | 9 participants, non-randomized, single group assignment, three arms, open label. | To evaluate the safety and efficacy of *E. kansui* extract in HIV-infected antiretroviral therapy (ART)-suppressed individuals. | Phase 1 | Not yet recruiting | CT02531295 |
| *T. wilfordii Hook F (*TwHF*),* China | 23 participants, parallel, non-randomized, two arms, open-label trial. | To evaluate the impact of TwHF on T-cell immune activation and immune response activation in HIV-infected immunological non-responders. | Not applicable | Completed | NCT02002286 |
| *TwHF,* China | 60 participants, randomized, parallel assignment, two arms. | To evaluate the efficacy and safety of TwHF in immune non-responders with HIV-1 infection. | Phases 1 and 2 | Unknown | NCT01666990 |
| Immunity 1 (Fuzheng 1), China | 60 participants, randomized, parallel assignment, two arms, double-blind, placebo-controlled trials. | To evaluate the effect of Immunity 1 (Fuzheng 1) on immune reconstitution in HIV patients. | Not applicable | Unknown | NCT00974285 |

**Table 3** Continued

| Medicinal plant and country | Study design | Purpose | Study phase | Recruitment status | ClinicalTrials.gov number |
|---|---|---|---|---|---|
| Immunity 1 and Immunity 3 (Fuzheng 1 and 3): composed of herbs with tonic and detoxification functions, China | 180 participants, randomized, parallel assignment, double-blind, placebo-controlled clinical trials. | To evaluate the effect of the combination of traditional Chinese medicine (TCM) and highly active antiretroviral therapy (HAART) on immune reconstitution in HIV/AIDS patients. | Not applicable | Unknown | NCT00974519 |
| Immunity 2 (Fuzheng 2), China | 180 participants, randomized, parallel assignment, two arms. | To research the effect of TCM on immune reconstitution in HIV/AIDS patients after HAART. | Not applicable | Unknown | CT00974454 |

In this regard, medicinal plants are being investigated as novel resources for the production of valuable and active secondary metabolites for the treatment of TB (Sharifi-Rad *et al.*, 2017). Different medicinal plants have been reported to exert potential effects against latent and/or drug-resistant forms of TB, intracellular *TB*, dormant bacilli (Table 2), and against HIV-TB co-infection, and some of them also exhibited immunomodulatory effects (Gupta *et al.*, 2017).

### *Synergistic antimycobacterial effects of plants*

Due to administration of two or more drugs concomitantly leads to the emergence of resistant circulating strains and the development of new drugs is a long process, it would be useful to identify adjuvants capable of boosting the efficacy of antibiotics against resistant strains. Mutations in the genomic targets of drugs, the production of drug-modifying and drug-inactivating enzymes, low cell wall permeability and efflux-related mechanisms have been identified as the main mechanism of resistance in *Mtb* (Gupta *et al.*, 2006). In this context, the susceptibility of microbes to antibiotics is influenced by the use of natural products (Ge *et al.*, 2010). *In vitro* synergistic effects induced by a combination of plant agents with antibiotics have been investigated (Table 4). The available reports on the synergy between plant compounds and antibiotics against *Mtb* strains present the feasible impact of these agents on restoring the susceptibility of resistant bacteria to antibiotics. A checkerboard synergy assay has been applied for the determination of synergistic activity and is generally defined by fractional inhibitory concentration index (FICI) values of $\leq 0.5$, antagonism by FICI values of $> 4.0$ and no interaction by FICI values from 0.5 to 4.0 (Odds, 2003). Finally, it should be noted that the validation of efficacy and safety of plant compounds as anti-tuberculosis agents takes a long time and, then in this case, more high-level randomized clinical trials are urgently needed (Sharifi-Rad *et al.*, 2017).

### *Search for plants with both anti-TB and anti-HIV activity*

Because the number of CD4 + lymphocytes is decreased in people infected with HIV, these individuals are also susceptible to acquiring or reactivating tuberculosis disease (Flynn and Chan, 2001). The treatment programmes for patients co-infected with HIV/TB encounter some barriers, such as intolerance, contraindications and poor adherence to medication regimes due to large number of medications. For example, for patients infected with HIV virus, the administration of 20 pills per day (1 non-nucleoside + 1 nucleoside type) is recommended for HAART, and 10–12 pills are recommended as monotherapy for TB (Dean *et al.*, 2002). Additionally, HIV-1 protease inhibitor drugs can neutralize the effect of rifampin administered for TB treatment (Driver *et al.*, 2001; Schwander *et al.*, 1995). Moreover, overlapping toxicity profiles may cause interruption or alteration of TB and HIV regimens which potentially resulting in microbiological or virological failure (Dean *et al.*, 2002). Therefore, the discovery and development of new agents with remarkable activity against HIV and *Mtb* are urgent requirements. In this regard, the isolation, purification and identification of valuable secondary metabolites from medicinal plants could be a promising, efficient and affordable approach for the control and treatment of both targets.

Naturally, anti-HIV(+)-calanolide A, a secondary metabolite extracted from *C. lanigerum*, exhibited activity against all strains of *Mtb* (MIC: 8–16 μg/mL) (Xu *et al.*, 2004). This compound induced rapid prevention of RNA and DNA synthesis followed by an inhibition of protein synthesis. Moreover, the ethanol extract of *Annona muricata* showed inhibitory activity against both *Mtb* with an MIC value of 125 μg/mL and HIV-1 integrase activity with IC50 < 100 μg/mL (van de Venter *et al.*, 2014). However, *A. muricata* extract exhibited cytotoxicity against Chang liver and HepG2 cells with IC50 values of 30 and 77 μg/mL, respectively. Recently, the effect of Mexican Julianaceae and Clusiaceae crude plant extracts against both HIV-RT and Mtb H37Rv has been investigated (Gómez-Cansino *et al.*, 2015). Among 14 Julianaceae species, three species, *Amphipterygium glaucum, A. molle* and *A. simplicifolium*, exhibited inhibitory activity against *Mtb* with IC50 values of 1.87–2.35 μg/mL and against HIV reverse transcriptase with IC50 values of 59.25–97.83 μg/mL. Among this group, *A. adstringens* was reported as a promising source of anti-TB agents because the stem bark extract prevented 95% of *Mtb* growth at a concentration of 50 μg/mL. Moreover, three species of Clusiaceae, namely *Vismia baccifera, Calophyllum brasiliense* and *Vismia Mexicana,* inhibited *Mtb* with IC50 values of 3.02–3.64 μg/mL and HIV reverse transcriptase with IC50 values of 26.24–35.17 μg/mL (Gómez-Cansino *et al.*, 2015). Among these three most active plants, *C. brasiliense* is more interesting because its leaves synthesize dipirano-tetracyclic coumarins, such as inophyllums, calanolides A, B and C, and soulatrolide, which were previously shown to exhibit activity against HIV-1 reverse transcriptase (Gurib-Fakim, 2006) and *M. tuberculosis* (Xu *et al.*, 2004).

**Table 4** Synergistic activity of medicinal plant with anti-TB drugs. (Gupta *et al.*, 2017)

| Plant names | Agents | Antibiotic | Fold reduction in MIC of anti-TB drug | FIC index | References |
|---|---|---|---|---|---|
| *E. natalensis* | 7-methyljuglone | Isoniazid | 4–6 | 0.2 | Bapela *et al.* (2006) |
| | | Rifampicin | | 0.5 | |
| *Galenia africana* | (E)-3,2′,4′-trihydroxy-3′-methoxychalcone, (2S)-5,7,2′-trihydroxyflavanone | Isoniazid1 | 16 | 0.12 | Mativandlela *et al.* (2009) |
| | | Isoniazid | 4 | 0.5 | |
| Commercial source (plant origin) | oleanolic acid (OA) | Isoniazid | 4–16 | 0.121–0.347 | Ge *et al.* (2010) |
| | | Rifampicin | 8–16 | 0.113–0.168 | |
| | | Ethambutol | 4–16 | 0.093–0.266 | |
| *Piper nigrum L.* | Trans-trans isomer of 1-piperoyl-piperidine (piperine) | Isoniazid | 8 | 0.25 | Sharma *et al.* (2010) |
| | | Rifampicin | 4–8 | <0.5 | |
| *Knowltonia vesicatoria (L.f.) Sims* | Trans-trans isomer of 1-piperoyl-piperidine (piperine) | Isoniazid | 8 | 0.25 | Labuschagné *et al.* (2012) |
| *Notopterygium incisum* | Isoimperatorin (IO) | Isoniazid | 4–6 | 0.2 | Guo *et al.* (2014) |
| | | Rifampicin | 5–20 | 0.133–0.472 | |
| *Mutellina purpurea L.* | Bisabolol | Rifampicin | 16 | 0.0625–0.125 | Sieniawska *et al.* (2015) |
| | (R)-limonene | | | 0.125–0.25 | |

## Current approaches towards production of anti-HIV and TB agents isolated from plant secondary metabolites

Crude extraction and/or isolated compound from the source plants evaluate for their anti-HIV and TB properties. However, these plants may have not been domesticated and their population maybe limiting. Additionally, the slow growth rate of source plants may significantly decrease the production of active natural products. The low concentration of active molecules in source plants as well as poor recovery of such compounds after extraction also contributes to production challenges. Then, the large scale of the source plants biomass is required to meet the demands of drug manufacturing. This scenario could cause massive damage on plant species and dramatically accelerate their extinction. Therefore, naturally available production capacity for compounds with anti-HIV and anti-TB activity is not sufficient to meet the demands of commercial development. In this context, plant cell cultures have emerged as sustainable and controllable technology for commercial production of active molecules. Advances in cell line selection, medium optimization, biotransformation, feeding precursors, hairy root cultures, product secretion, cell permeabilization and scale-up increased plant natural products yield (Habibi *et al.*, 2017a, 2018a). The development of efficient, strong and novel 'omics' technologies such as next-generation sequencing intensified plant cell culture system and in this regard the number of companies using plant cell cultures for the production of active natural products continues to rapidly expand (Ochoa-Villarreal *et al.*, 2016). This technique has been successfully used for the production of calanolides in a callus culture of *C. brasiliense* (Bernabé-Antonio *et al.*, 2010) and also artemisinin in hairy root cultures of *Artemisia annua L.* (Ahlawat *et al.*, 2014). Moreover, the emergence of cambial meristematic cells could significantly circumvent potential difficulties related to low yields, instability of product yield, a relatively slow growth rate, industrial scale-up and downstream processing (Ochoa-Villarreal *et al.*, 2015). This technology has been implemented for the production of ginsenosides with yield of 268 mg/kg (Lee *et al.*, 2010). Previously, the anti-HIV effect of ginsenosides alone or in combination with zidovudine monotherapy or HAART therapy has been demonstrated (Im *et al.*, 2016).

Beside these advances, progress in genome manipulation resulted in relatively large quantities of pharmaceuticals. Metabolic engineering is a promising strategy to obtain adequate active compounds for industrial applications. Since, this technology is related to the modification of cellular activities through the manipulation of enzymatic, transport and regulatory functions of the cell, those bottlenecks associated with commercial production of active compounds such as slow growth of plant, genetic instability, low concentration of end products, geographical position and climatic conditions can be removed. Metabolic engineering can boost productivity through increasing the cell number production and improve the carbon flux by a biosynthetic pathway via overexpression of genes. The main advantage of this strategy is that it can offer a continuous and reliable source of active secondary metabolites. In the metabolic engineering for the production of plant secondary metabolites, identification of the biosynthetic pathways of desired compounds, rate-limiting steps and enzymes involved in reaction is required (Hussain *et al.*, 2012). However, the present knowledge in biosynthetic pathways in plants is often still in its infancy and then development of strategies based on cellular and molecular information is imperative. Moreover, the emergence of novel techniques of molecular biology, so as to produce transgenic cultures and also to impact the expression and regulation of biosynthetic pathways, is one of the critical steps towards making plant cell culture as a sustainable and reliable source for commercial production of active compounds.

## Plant molecular pharming related to HIV and TB

### Advantages and obstacles

In the past two decades, plant genetic engineering technologies have shown substantial progress for the production of pharmaceutical products. Plant-based pharmaceutical production has gained specific attention due to numerous advantages that are equivalent or more beneficial compared with those of standard expression systems (Daniell *et al.*, 2015; De Martinis *et al.*, 2016).

 

Like yeast and mammalian cells, plants have the cellular machinery needed to promote the post-translational modifications required to glycosylate and fold proteins with the highest fidelity, and unlike mammalian and bacterial cells, plants are devoid of human pathogens and bacterial endotoxins. Additionally, plants provide high biosynthesis capacity with low production cost in the form of fresh leafy cells and edible seeds. The latter offer a further benefit for the stable storage and transportation of target proteins over long periods of time as well as high protein accumulation (Vamvaka *et al.*, 2016a, 2018; Yao *et al.*, 2015). In contrast, the downstream processing costs from traditional systems remain a key concern in the production of recombinant proteins, and edible plants can eliminate the need for these costly purification processes as well as for cold chain processes for transport and storage (Chan and Daniell, 2015; Kwon *et al.*, 2013a). Thus, plant-derived drugs can be produced efficiently in developing countries without the need for expensive cold chain and transport processes, which place an immense burden on the healthcare system.

Two general strategies are available for expression of HIV and TB recombinant proteins including (i) transient expression system using viral or bacterial vector or combinations of therefore and (ii) transgenic plants via nuclear or plastid expression. Compared to expression of recombinant proteins in standard systems which restricted to some specific host systems (e.g. *E. coli*, *Pichia pastoris* and CHO cells), a range of plant species and different expression strategies have been developed by academic and industrial teams for the production of recombinant proteins. Tobacco and its close relative *N. benthamiana* are widely used for transient and stable production of recombinant proteins. In the term of biomass production, it is estimated that high biomass yields of up to 100 000 kg per hectare in open filed (Conley *et al.*, 2011) and 180 000 kg per year by vertical farming unit (VFU) with a footprint of ~6500 $m^2$ including ~2000 $m^2$ (Holtz *et al.*, 2015) could be achieved by tobacco platform. *N. benthamiana* also is the bioreactor of choice for large-scale production of recombinant proteins in short period of time. Recently, by the Caliber Biotherapeutics facility, it is feasible to grow more than 4 million *N. benthamiana* plant at a single time in highly automated multilevel growing environment under proprietary LED lighting fixtures with biomass production per week ranged from 3500 to 7000 kg (Holtz *et al.*, 2015). Both of these plant platforms have more substantial technical benefits over mammalian cell-based system including high-throughput screening and development of target recombinant protein, available simple process for both primary amino acid manipulation and glycosylation engineering, and low-cost process for large-scale production up to 150 kg of purified product per year (Holtz *et al.*, 2015). The presence of nicotine as a toxic alkaloid may restrict its oral therapeutic applications; however, there are reports on the development of new varieties of tobacco with low alkaloid which are suitable for the production of recombinant proteins with the potential of direct oral administration of plant tissue or crude protein extracts (Joensuu *et al.*, 2008), although FDA has not yet approved any tobacco product for clinical applications. The use of edible plants for oral administration could remarkably remove prohibitively expensive fermentation, purification processes, delivery, cold storage and transportation. Expression systems also have been developed based on other plants such as rice, carrot, maize, Spinach, lettuce (Chan and Daniell, 2015; Lakshmi *et al.*,2013), cowpea, potato, Arabidopsis (Scotti *et al.*, 2010) and moss (Orellana-Escobedo *et al.*, 2015) for the production of recombinant HIV and TB antigens. Advantages and disadvantages of these systems are reviewed elsewhere by Habibi *et al.* (2017b).

Besides the establishment of scalable unit operations, downstream processes have been optimized for efficient and affordable production of recombinant proteins. Different protocols have been established for initial extraction of plant-based recombinant proteins. These protocols have been designed based on tissue and subcellular localization of accumulated protein. In the case of secretion of recombinant protein in extracellular compartments, protein recovery is easy, however encounter some challenges related to protein degradation by plant cell proteases. Recombinant proteins targeted to leaf or seeds organs are generally extracted by blade-based homogenizers/screw presses or mills, respectively (Buyel, 2018), following extraction buffer within the pH range 5.0–8.0. However, protein extraction process is eliminated when protein drugs bioencapsulated in plant cells are orally delivered (Daniell *et al.*, 2016a,b). In this context, transplastomic plants can produce much higher levels of transgene product (Boyhan and Daniell, 2011; Daniell *et al.*, 2016a,b; Kwon *et al.*, 2013a; Ruhlman *et al.*, 2010), enable delivery of higher/more concentrated doses. Lyophilization further decrease costs by increasing the concentration of therapeutic proteins, which reduces the amount of material needed per dose (Kwon *et al.*, 2013a,b; Lakshmi *et al.*,2013; Shil *et al.*, 2014; Su *et al.*, 2015a,b), allowing stable, long-term storage at ambient temperatures—sometimes for as long as two years—without loss of efficacy, thereby breaking the cold chain barrier (Kwon *et al.*, 2013a,b; Lakshmi *et al.*, 2013; Shil *et al.*, 2014; Su *et al.*, 2015a) and eliminating microbial contamination.

## Recombinant protein products for HIV

### *Broadly neutralizing antibodies*

Broadly neutralizing antibodies (bNAbs) are a rare class of antibodies that can develop in 15%–25% of people years after the original exposure (Teh *et al.*, 2014). Most bNAbs show effective activity against HIV virus by disrupting viral replication via immune exclusion or through Fc-mediated antibody activities (Overbaugh and Morris, 2012). In the case of HIV-1 infection, evidence from experimental animal models demonstrates that bNAbs can inhibit HIV-1 acquisition (Teh *et al.*, 2014). During recent years, the monoclonal antibody therapeutics market has grown significantly, that is it increased from $50 billion in 2010 to nearly $90 billion in 2015, and this growth is predicted to reach approximately $150 billion by 2021 (https://www.reportbuyer.com/product/5122216).

Mammalian cells are the gold standard expression system for the majority of FDA-approved therapeutic antibodies (Lalonde and Durocher, 2017). Given that the high potential of mammalian cells will not meet the demand, plants can be considered an alternative strategy for the production of therapeutic antibodies on a large economic scale. For instance, therapeutic mAbs for one million patients who need one gram of product can be produced in a 250 000 L fermenter (Ecker *et al.*, 2015). However, this may not meet the need of global diseases, such as HIV/AIDS. For HIV prevention, for example, a twice weekly dose of 30 mg mAb is recommended for women susceptible to HIV, and this amount would correspond to more than 3 tons of mAb per year for a million women (Buyel *et al.*, 2017). The demand for an efficient prophylactic approach could also escalate dramatically, equalling ~50-100 tons of mAb per year, if we include the HIV+ population (26 million in sub-Saharan Africa) and the at-risk

 

population in entire countries or regions. The production of such massive quantities of mAb with the currently used mammalian cell culture platforms may not be feasible. CHO cells can ultimately produce a maximum amount of 7.5 tons of mAb per year under continuous and optimal operation (Kelley, 2007). Moreover, the high investment cost required for CHO cell medium ($US 14–22 million per batch for medium alone) and reactor set-up as well as issues with contamination (Buyel *et al.*, 2017) are additional barriers that encourage the search for a cost-effective and scalable platform.

In contrast to fermentation systems, plants can be scaled efficiently and simply by sowing more seeds. In comparison with that of CHO cells, the media cost for a plant expression platform would be approximately $US 4.5 million assuming biomass yields and fertilizer consumption (Buyel and Fischer, 2012). Moreover, in the context of commercialization of process, the intrinsic yield (i.e. the accumulation of product per unit biomass) is not as crucial as the overall economy of the process (Fischer *et al.*, 2013). It means that upstream production in plant systems benefits from scalability. On the other hand, increasing in the number of plants producing target protein is not as expensive as doubling the capacity of a fermenter facility (Fischer *et al.*, 2013). Therefore, the production of mAbs in a plant platform appears to have benefits over a CHO cell fermenter in the upstream process; however, the downstream capture and purification steps are likely equivalent to those associated with CHO cells. The purification steps using the protein A capture step are similar for the manufacturing of all mAbs and typically include ion exchange or hydrophobic interaction chromatography (Ma *et al.*, 2015). Moreover, the costs associated with formulation, cold storage, transportation and sterile delivery are other concerns that increase the cost of the end products. In this context, economic models for commercial molecular farming based on seed technology and/or oral delivery of vaccine bioencapsulated in plant cells have been established to decrease the cost of recombinant protein in term of downstream process, cold storage requirements and sterile delivery.

### *Plant-based candidate vaccines against HIV*

Despite 30 years of efforts to develop vaccines for eliciting antibodies that can neutralize HIV-1 and confer protective immunity, there is no FDA-approved vaccine (Klein *et al.*, 2013; McCoy and Weiss, 2013). The high genetic variability of HIV and its capacity to escape the immune system resulted in failure of effective vaccine development (Cohen, 2009). Hundreds of clinical trials for HIV vaccine candidates have been established, but none of them exhibited efficient and sufficient effect on successfully neutralizing HIV to be approved for clinical use (Esparza, 2013; Fauci and Marston, 2015; Haynes and Mascola, 2017). Many attempts have been made to develop new candidate vaccines based on protein subunits, DNA vaccines, synthetic peptides, viral vectors expressing HIV-1 genes, and attenuated and inactivated virus (Gamble and Matthews, 2011). However, the last two approaches were unsuccessful as they showed poor protective efficacy. Several subunit vaccines have been developed based on immunogenic HIV chimeric proteins to elicit broad humuoral and cytotoxic CD8 + responses and to protect against HIV (Rosales-Mendoza *et al.*, 2012). Env complex (gp120 and gp140), Gag, Tat and Nef proteins were immunogenic, but Env complex is the only target protein, which can induce neutralizing antibodies (NAbs) inhibiting viral entry. However, Gag, Tat and Nef structures are able to elicit cellular responses, which are involved in viral load blocking (Klasse *et al.*, 2012; Mascola and Haynes, 2013; Rosales-Mendoza *et al.*, 2015).

Several HIV candidate plant-based vaccines targeting early HIV proteins and structural HIV proteins have been expressed in plants using transient and stable expression systems. These candidates are reviewed elsewhere (Rosales-Mendoza *et al.*, 2015). The combination of plant vaccine with other (or heterologous prime-boost vaccination) could be advanced to elicit T-cell responses. In this approach, the first vaccine is applied to 'prime' the elicitation of antigen-specific T cells, consisting of a set of memory cells, which endures beyond destruction of the antigen. On the other side 'boosting' vaccine, targeting the same antigen(s) contributes to elicit the extension of antigen-specific memory T cells (Webster *et al.*, 2005). Prime-boost vaccination strategy has been demonstrated to increase the rate of antigen-specific CD4 + and CD8 + T cells, improved high avidity T cells and consequently boosted protective efficacy against pathogen invasion (Dalmia and Ramsay, 2012; Shete *et al.*, 2014). Plant-derived vaccines are suitable for booster vaccines, specifically where the number of doses is needed over long periods to induce and maintain immunity, in population where reinfection occurs due to environmental exposure. Proof-of-concept reports described in mice have indicated the efficient use of plants cell in heterologous prime-boost strategies (Daniell, 2019; Daniell *et al.*, 2018; Lakshmi *et al.*, 2013; Meador *et al.*, 2017). However, in the absence of such priming, plant cells are not suitable for vaccination against infectious diseases (Chan *et al.*, 2016; Daniell, 2019; Xiao and Daniell, 2017).

Multi-epitope chimeric proteins are considered as promising candidates for the development of HIV vaccines (Vasan and Michael, 2012). In this context, the Env proteins gp120 and gp140 are the main targets for the establishment of neutralizing antibodies because these can bind to CD4 and its co-receptor on CD4 + T lymphocytes. The application of multi-epitope chimeric proteins enables viable immunization against non-immunodominant epitopes as well as the boosting of neutralizing antibodies targeting Env (Forsell *et al.*, 2009). The C4(V3)6 protein, a multi-epitopic antigen composed of several V3 variants together with the C4 domain of HIV, has been produced in both tobacco and lettuce plants (Govea-Alonso *et al.*, 2013a), and the oral administration of lettuce-derived C4(V3)6 elicited local and systemic immune responses in mice (Govea-Alonso *et al.*, 2013b), but HIV viral neutralization was not evaluated.

Recently, Orellana-Escobedo *et al.* (2015) expressed multi-epitope HIV proteins comprising epitope sequences from gp120 (C4 and V3) or four variants of the ELDKWA epitope from gp41 in moss (*Physcomitrella patens*) plant and demonstrated that this moss-derived HIV antigen is immunogenic in mice when subcutaneously administered and elicits humuoral responses against the ELDWKA epitopes. However, neutralization of HIV was not evaluated.

### *Lectins*

Lectins such as griffithsin (GRFT), cyanovirin-N (CV-N) and actinohivin (AH) are potential microbicide candidates against the sexual transmission of HIV-1 which shows exquisite specificity to high-mannose-type glycans on the viral envelope (Env) as well as nanomolar neutralizing activity (O'Keefe *et al.*, 2009, 2015; Tanaka *et al.*, 2009). These lectins have been produced by different heterologous systems such as bacteria and yeast, however, faced critical drawbacks such as inclusion body

production following expensive and laborious re-solubilization and refolding process by bacteria (O'Keefe *et al.*, 2009). Hyperglycosylation by yeast resulted in protein dimerization and loss of activity (Mori *et al.*, 2002), and also the higher intrinsic cost related to large-scale production. Additionally, the success of lectin-based commercial products as topical and parenteral microbicides for HIV depends on a bulk production system that can provide high quantities of high-quality microbicides for formulation and characterization (Fuqua *et al.*, 2015). Upstream and downstream processes as well as analytics programmes are related to manufacturing costs in current good manufacturing practice (cGMP) due to the need for quality control. These costs can affect the overall cost of lectin manufacturing because these microbicides are mostly required in resource-poor countries with a high risk of HIV exposure. Consequently, these lectins, as a microbicide, should be a low-cost recombinant protein (Fuqua *et al.*, 2015). In this context, transgenic plants are ideal systems for low-cost and scalable production of recombinant proteins with the potential for the direct preparation and application of crude extracts (Stoger *et al.*, 2014). Cereals such as rice and soybean are efficient platform for the production of microbicides as they allow high-level production of proteins in desiccated tissue, which therefore intensify stability during transport and storage, and seed extract inherently regarded safe as it is staple food crop (Sabalza *et al.*, 2013). In the term of downstream process, the ability of production of protein body by cereals is a benefit as protein bodies are insoluble in low-salt aqueous buffers, and they can be easily purified by centrifugation. Particularly, this advantage could be expanded by the production of recombinant proteins by rice endosperm. Despite to other cereals, the presence of two different protein bodies in rice endosperm enables expression of different proteins simultaneously and facilitates their recovery by first separating the compartments via density centrifugation (Vamvaka *et al.*, 2016a). This technology has been implemented for the production of GRFT (223 μg/g dry seed weight) and CV-N (10 μg/g dry seed weight) by Vamvaka *et al.* (2016a,b). However, in contrast to other cereals such as maize, wheat and barley, the content of rice seed is much lower, and the cost related to growing is much higher.

Transient expression system has also been used for large-scale production of lectins. For example, GRFT has been expressed transiently in *N. benthamiana* using a vector based on *tobacco mosaic virus* (TMV) resulted in the production of up to 1 g of soluble GRFT per kg of fresh leaf weight (O'Keefe *et al.*, 2009). Previously, the production of GRFT has been reported in *E. coli* with a yield of 819 mg/L, but only 66% of the protein was present in the soluble fraction and the 34% was found in inclusion bodies and was impossible to re-solubilize even using detergents. However, the high cost of in vitro RNA transcription used in transient expression of GRFT is a notable problem of virus-based systems, and the purification of GRFT based on tobacco mosaic virus (TMV) is considered to result in some contamination with TMV coat protein and protein degradation, which would result in the need for further purification steps (Habibi *et al.*, 2018b). We recently reported our efforts to optimize high-level expression and purification processes for the production of GRFT based on a non-viral expression vector, and our proposed process was able to improve the recovery of GRFT using a one-step protocol for purifying GRFT from crude extract (Habibi *et al.*, 2018b). This is comparable to GRFT produced in *E. coli* after refolding, but neither re-solubilization nor refolding steps are needed.

Recently, the new approach of combining neutralizing antibodies with lectins as bispecific anti-HIV proteins against HIV diversity has been developed. The wide diversity and high mutation rate of the virus enable virus to evade monotherapy rather quickly (De Mendoza, 2016). However, an optimal combination therapy should consist of individual anti-HIV-1 component that synergistically works together and efficiently inhibit the virus replication. In the last decades, several bispecific anti-HIV agents have been produced and have been shown to have ability to neutralize several strains (Bournazos *et al.*, 2016; Huang *et al.*, 2016). In this context, the lectin actinohivin, extracted from actinomycete bacteria, has been shown as a promising candidate with high anti-HIV activity (Matoba *et al.*, 2010). The production and anti-HIV activity of one of such bispecific entry inhibitors consisting of Avaren fused to the bNAb VRC01 were reported *recently* (Kasinger *et al.*, 2019). According to this model, neutralizing activity against HIV strain was enhanced. However, compared to the parental molecules (~150 mg/kg for VRC01 (Hamorsky *et al.*, 2013) and up to 100 mg/kg for Avaren (Kasinger *et al.*, 2019)), fused VRC01Fab-Avaren yield was lower and required three-step chromatography purification procedure. Similarly, the combination therapy using three microbicidal proteins 2G12, the GRFT and CV-N cocktail produced in rice endosperm (Vamvaka *et al.*, 2018). Extracts of transgenic plants expressing all three proteins demonstrated increased in vitro binding to gp120 and synergistic HIV-1 neutralization. Besides enhanced neutralizing activity, application of crude seed extract directly can significantly eliminate expensive purification process as the deployment of antibodies and lectins as HIV-1 entry inhibitors is critically contingent on large-scale inexpensive production.

## Plant-derived vaccines against TB

Vaccines are the most efficient, affordable and least invasive strategy to combat TB. Numerous efforts have focused on the design and development of new candidates as anti-tuberculosis vaccines. Approximately 25 novel and potent TB vaccine candidates are in development and/or different stages of clinical trials (http://www.tbvi.eu/what-we-do/pipeline-of-vaccines/) (Table 5). Most of these vaccines are subunit vaccines, whereas others are considered attenuated live TB vaccines. Compared with attenuated live TB vaccines, subunit vaccines offer more advantages in terms of safety and efficacy as well as standardization (Agger and Andersen, 2001). Subunit vaccines can be categorized into two groups: primary prevention vaccines (aiming to replace the Bacillus Calmette-Guerin vaccine (BCG)) and booster vaccines (intended for revaccination). The booster vaccines are required to inhibit reactivation of the pathogen during latent tuberculosis (Uvarova *et al.*, 2013). ESAT-6, CFP10, Ag85B, MTB72F and LipY are promising subunit candidates designed from surface components and secreted proteins of TB (Lakshmi *et al.*, 2013; Uvarova *et al.*, 2013). The latter is best suited for vaccine production because antigenic proteins are actively secreted during the early phase of the TB life cycle and can thus promote a strong T-cell response and $\gamma$-interferon secretion (Ben Amor *et al.*, 2005; Brodin *et al.*, 2004; Gao *et al.*, 2009). In general, any mycobacterial antigen that induces both CD4 and CD8 T cells and confers protective immunity is a promising candidate for subunit vaccination against TB.

The establishment and development of a potent vaccine that boosts the immune response to tuberculosis at the mucosal level is a challenge in the field of vaccine research. Previous studies

 

**Table 5** TB vaccines in development

| Agent and sponsor | Study design | Purpose | Class/type | Study phase | Recruitment status | ClinicalTrials.gov number and/or references |
|---|---|---|---|---|---|---|
| Vaccae™, Anhui Zhifei Longcom Biologic Pharmacy Co., Ltd | Randomized, parallel assignment, double, two arms | Evaluation of efficacy of Vaccae to prevent tuberculosis in high-risk groups of tuberculosis infection. | Whole-cell *M. vaccae* | Phase III | Completed | NCT01979900 |
| VPM1002, Serum Institute of India Pvt. Ltd | 2000 participants, randomized, parallel assignment, double-blind | To evaluate the efficacy and safety of VMP1002 in the prevention of tuberculosis recurrence in pulmonary TB patients after successful TB treatment in India. | Live rBCG | Phase II/III | Recruiting | NCT03152903 |
| M72/AS01E, Aeras, GlaxoSmithKline | 3253 participants, cohort, two arms | Collection and storage of biological samples for evaluation of correlates of tuberculosis. | Protein/adjuvant subunit vaccine | Phase II | Completed | NCT02097095 |
| DAR-901, Dartmouth-Hitchcock Medical Center | 650 participants, randomized, parallel assignment, placebo-controlled, double-blind | Prevention of infection with mycobacterium tuberculosis among adolescents who have previously received BCG. | Whole-cell *M. obuense* | Phase II | Active, not recruiting | NCT02712424 |
| H4:IC31, Aeras | 990 participants, randomized, parallel assignment, placebo-controlled, partially blinded | To evaluate safety, immunogenicity and prevention of infection with mycobacterium tuberculosis of AERAS-404 and BCG revaccination in healthy adolescents. | Protein/adjuvant subunit vaccine | Phase II | Completed | NCT02075203 |
| H56:IC31, Aeras | 98 participants, randomized, parallel assignment, four arms, double-blind | To evaluate the safety and immunogenicity of AERAS-456 in HIV-negative adults with and without latent tuberculosis infection. | Protein/adjuvant | Phase II | Completed | NCT01865487 |
| RUTI®, Archivel Farma S.L. | 95 participants, randomized, parallel assignment, double-blind | To assess the safety, tolerability and immunogenicity of two doses of RUTI® vaccine administered four weeks apart after one month pre-treatment with INH. | Fragmented MTB | Phase II | Completed | NCT01136161 |
| ID93 + GLA-SE, Infectious Disease Research Institute(IDRI) | 60 participants, randomized, parallel assignment, double-blind, | To evaluate the safety and immunogenicity of the ID93 + GLA-SE vaccine in HIV-uninfected adult to patients after treatment completion. | Protein/adjuvant | Phase IIa | Completed | NCT02465216 |
| MTBVAC, Biofabri, S.L | 99 participants, randomized, sequential assignment | To evaluate the safety, reactogenicity, immunogenicity and potential for IGRA conversion and reversion, of MTBVAC in South African newborns. | Live genetically attenuated MTB | Phase II | Not yet recruiting | NCT03536117 |
| Vaccine (692342), GSK | 302 participants, randomized, parallel assignment | To assess safety and immunogenicity study of GSK biologicals' candidate tuberculosis vaccine (692342) when administered to healthy infants. | Fusion protein | Phase II | Completed | NCT01098474 |
| MVA85A, University of Oxford | 37 participants, randomized, parallel assignment, open label | To evaluate mucosal administration of a candidate TB vaccine, MVA85A, as a way to induce potent local cellular immune responses and avoid anti-vector immunity. | Viral vector | Phase I | Completed | NCT01954563 |

 

**Table 5** Continued

| Agent and sponsor | Study design | Purpose | Class/type | Study phase | Recruitment status | ClinicalTrials.gov number and/or references |
|---|---|---|---|---|---|---|
| TB/FLU-01L, Research Institute for Biological Safety Problems | 36 participants, randomized, parallel assignment, double-blind | To evaluate the safety and immunogenicity of 2 doses (Day 1 and Day 21) TB/FLU-01L tuberculosis vaccine in BCG-vaccinated healthy adult subjects aged 18-50 years. | Viral vector | Phase I | Completed | NCT03017378 |
| TB/FLU-04L, Research Institute for Biological Safety Problems | 36 participants, randomized, parallel assignment, double-blind | To explore the safety and immunogenicity of 2 doses (Day 1 and Day 21) TB/FLU-04L tuberculosis vaccine versus matched placebo in BCG-vaccinated healthy adult subjects aged 18-50 years. | Viral vector | Phase I | Completed | NCT02501421 |
| ChAdOx1 85A + MVA85A, Oxford University | 42 participants, randomized, parallel assignment, open label | To evaluate the safety and immunogenicity of a ChAdOx1 85A vaccination with and without MVA85A boost in healthy BCG-vaccinated adults. | Viral vector | Phase I | Completed | NCT01829490 |
| Ad5Ag85A, McMaster University, CanSino | 24 participants, non-randomized, single group assignment, open label | To evaluate the safety and immune responses of Ad5Ag85A, administered to healthy volunteers. | Viral vector | Phase I | Completed | NCT00800670 |
| MVA85A-IMX313, University of Oxford | 30 participants, randomized, parallel assignment | To evaluate the safety and immunogenicity of MVA85A-IMX313 compared to MVA85A in BCG-vaccinated adults. | Viral vector | Phase I | Completed | NCT01879163 |
| GamTBvac, Gamaleya Research Institute of Epidemiology and Microbiology, Health Ministry of the Russian Federation | 60 participants, randomized, single group assignment | Evaluation of the safety and immunogenicity of the 'GamTBvac' against the tuberculosis. | Subunit vaccine | Phase I | Completed | NCT03255278 |
| AERAS-404 (HyVac4), Aeras | 60 participants, randomized, parallel assignment, double-blind | To evaluate safety and immunogenicity of AERAS 404 administered in adults. | Protein/adjuvant | Phase I | Completed | NCT02074956 |
| H64 + CAF01, Statens Serum Institut | – | To investigate which epitopes are immunodominant during TB infection and if removal of the dominant epitopes will allow for exposure of cryptic/subdominant epitopes and improved protection. | Protein/ adjuvant | Preclinical | – | (https://www.ssi.dk/) |
| rBCGΔais1/zmp1, University of Zürich, Aeras | – | Global boosting and to safely replace BCG in the (HIV-exposed) newborns. | Recombinant BCG | Preclinical | – | Sander *et al.* (2015) |
| ChAdOx1.PPE15, University of Oxford, TBVI | – | To investigate protective efficacy of BCG in guinea pigs | Viral vector | Preclinical | – | (http://www.tbvi.eu/what-we-do/pipeline-of-vaccines/) |

 

**Table 5** Continued

| Agent and sponsor | Study design | Purpose | Class/type | Study phase | Recruitment status | ClinicalTrials.gov number and/or references |
|---|---|---|---|---|---|---|
| CysVac2/Advax, University Sydney, TBVI | – | To investigate the ability of the vaccine to protect in additional animal models (e.g. guinea pigs) and define efficacy in post-exposure models, in order to strengthen the case for clinical development of the vaccine. | Protein/adjuvant | Preclinical | – | Counoupas *et al.* (2017) |
| HBHA, Institut Pasteur Lille, Aeras, TBVI | – | To investigate the immunological response and protection of mice immunized with HBHA formulated in lipid-containing nanoparticles and adjuvanted with CpG, a TLR9 ligand. | Protein/adjuvant | Preclinical | Completed | Verwaerde *et al.* (2014) |
| Therapeutic vaccine –MVA platform, Transgene SA, TBVI (https://www.transgene.fr/) | – | To improve treatment of TB, in particular linked to DR (drug resistant) strains, and to prevent reactivation and/or reinfection in the adult DS (drug sensitive) population, in particular from endemic countries. | Viral vector | Preclinical | – | Leung-Theung-Long *et al.* (2015) |
| rCMV, Louis Picker of Oregon Health and Science University, AERAS. | – | To study safety concerns while maximizing the vaccine's protective potential. | Viral vector | Finishing preclinical development | – | (http://www.aeras.org/candidates) |

BCG, Bacille Calmette-Guérin; ChAd, chimpanzee adenovirus vector; GSK, GlaxoSmithKline; HBHA, heparin-binding haemagglutinin; MTB, *Mycobacterium tuberculosis*; MVA, modified vaccinia virus Ankara; rCMV, recombinant cytomegalovirus; TB, tuberculosis; TBVI, TuBerculosis Vaccine Initiative.

 

have shown that novel mucosal administration strategies can definitely boost vaccine efficacy due to local immuno-protective responses at the respiratory system mucosa (TBVI Organization. Available from: www. tbvi.eu/about-us/organization/ clinicaldevelopment-team.html [Last accessed 12 September 2014]). Thus, mucosal immunization strategies can remarkably contribute to the establishment and development of improved vaccines. However, vaccine formulations remain challenging due to the high costs associated with antigen expression and purification. Efficient and affordable vaccination approaches should rely on low-cost production systems with facile delivery, which will significantly facilitate massive immunization in limited recourse settings, where the vaccine is urgently required (Rosales-Mendoza *et al.*, 2015). Different systems have been used for delivery of subunit vaccines including recombinant bacterial vector system (Triccas, 2010), recombinant viral vector system (Sereinig *et al.*, 2006) and lipoglycan–protein conjugate system (Hamasur *et al.*, 2003). However, the high cost of these systems, as well as concerns related to patient safety particularly in terms of bacterial (Yurina, 2018) and viral vectors, promoted plant-based delivery system approach. By using the plant cell as the carrier, subunit vaccines can be delivered through mucosal route such as oral administration. The mucosal route is ideal because it is non-invasive and more favourable. Moreover, oral vaccination routes do not need special skills and remove disadvantages related to syringe-based injection or other required invasive procedures. As *Mtb* is a respiratory pathogen, vaccination through mucosal route can elicit both mucosal and systemic immune system responses (Cong *et al.*, 2014). Additionally, bioencapsulation of subunit vaccine into plant cell is advantageous as plant cell protect antigens from harsh environments and enzymatic reactions in the gut (Arlen *et al.*, 2008).

However, antigen entry from the intestine to the immune or circulatory system needed fused carrier proteins such as *E. coli* cholera toxin B (CTB) subunit (Limaye *et al.*, 2006; Xiao *et al.*, 2016). Functional CTB is pentameric and interacts with GM1 ganglioside, which is available in membranes of intestinal epithelial cells, neurons and immune cells, among others (Limaye *et al.*, 2006). It has been demonstrated that the adjuvanticity of CTB enhances when fused with foreign antigens. This could potentiate better uptake of antigen across the mucosal linings and provide sufficient antigen for dendritic cells and macrophages and then boost systemic and mucosal immune response. This concept has been used for range effective subunit booster vaccine in plant system, and results showed protective immunity against disease states (Arlen *et al.*, 2008; Daniell, 2019; Daniell *et al.*, 2018; Davoodi-Semiromi *et al.*, 2010; Ruhlman *et al.*, 2007a). Following priming with BCG, oral administration of plant-based booster vaccines is efficient strategy to elicit mucosal as well as systemic immunity. Chloroplast-derived vaccine candidate including CTB-ESAT6, CTB-Mtb72F and LipY fusion protein has been efficiently used in oral boost treatment regimen (Lakshmi *et al.*, 2013).

Critical factors involved in practical development of oral plant vaccines include adequate level antigen production, for immunization, stability, storage and vaccine efficacy. In relation to antigen expression, tobacco plants are ideal platform for large-scale production. It was reported that 80 mg of CTB-ESAT6 can be produced from a single tobacco plant and a total of 1.92 kg can be obtained from an acre of land based on three cuttings in a year (Lakshmi *et al.*, 2013). For some commercial tobacco cultivars with high-level production, antigen yield is predicted to be much higher. As described above, cost of end product by oral administration of edible plant-based subunit vaccines would be much lower. Based on human trails, it was demonstrated that 50 μg of subunit vaccine ESAT-6 is required to promote strong and long-lasting *Mtb* specific T-cell responses. The expression of CTB-ESAT6 following lyophilization resulted in increasing of antigen to 249 μg/g of lyophilized leaves, requiring only 200 mg plant powder for oral immunization to maintain protective immune response. Lettuce plants were chosen as an alternative system to tobacco plant for the production of TB antigen candidate as they are leafy vegetables and have suitable properties. Several vaccine antigens and biopharmaceuticals have been expressed and validated in transplastomic lettuce plants (Daniell, 2019; Daniell *et al.*, 2016a,b; Su *et al.*, 2015a).

## Conclusion

TB and AIDS are high-burden diseases that lead to death worldwide and place a major burden on the public health system, particularly in resource-limited settings. Combined with the growing population in developing countries with high burden of HIV and TB, this will increase the demand for anti-HIV and TB agents. Biotechnological processes are required to supply such agents, but established production systems such as those based on microbes and mammalian cell cultures are unlikely to fulfil expectations related to this urgent need because of the expensive production costs and the inherent complexity and toxicity of anti-HIV and TB agents. In this context, plants provide an efficient and affordable platform to fill this gap because they can produce a large number of agents with anti-HIV and TB activity. Plant natural product and/or plant extracts with killing potential are promising approaches to tackle the prevention and control of both diseases. Some plants have been shown to be promising sources of anti-TB and HIV drugs because they effectively inhibit both pathogens at very low concentration, which is important in terms of drug-related toxicity during long-term treatment. However, isolation, purification and identification of target compounds from complex crude extracts are complicated. The production of materials with uniform levels of active ingredients in native plants is a major challenge. Moreover, the validation of the efficacy and safety of plant-based compounds is the most important issue, which needs rigorous clinical evaluation.

Using plants as vaccine biofactories and orally administration by edible plants have been shown as a promising strategy to elicit both systemic and mucosal immune responses. However, parenteral formulations require extensive purification and expensive cold storage/transportation. In contrast, orally administration can be obtained by convenient lyophilization of plant biomass expressing the target protein. Methods are readily available for growth of plants in FDA-approved hydroponic growth facilities, lyophilization, drug dose determination, evaluation of stability upon long-term storage at ambient temperature, oral delivery of antigens to the immune system and evaluation of immune titres and protection against pathogen or toxin challenges. However, orally delivered plant antigens without injectable priming results in inducing tolerance rather than immunity (Daniell *et al.*, 2016a, b; Herzog *et al.*, 2017; Su *et al.*, 2015a,b). Therefore, vaccine antigens made in plant cells can only function after injectable priming (Chan *et al.*, 2016; Daniell, 2019; Xiao and Daniell, 2017). This approach is suitable where injectable vaccine is not affordable for a large majority of global population and a low-cost booster vaccine is needed (like polio booster vaccine). In the

 

context of HIV, there is no approved injectable vaccine and therefore boosting via plant cells is not currently feasible. However, oral boosting with antigens made in plant cells against TB after BCG vaccine priming is feasible and beneficial because of generation of both mucosal and systemic immunity. Boosting is urgently needed to maintain immunity in global regions where reinfection rate is very high and mucosal immunity conferred by oral antigens is essential to deal with pathogen at the site of entry.

## Conflict of interest

The authors declare no conflict of interest.

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